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Research article

Compound-specific carbon isotope patterns in needles of conifer tree species from the Swiss National Park under recent climate change

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ABSTRACT

Elevated CO_2 along with rising temperature and water deficits can lead to changes in tree physiology and leaf biochemistry. These changes can increase heat- and drought-induced tree mortality. We aim to reveal the impacts of climatic drivers on individual compounds at the leaf level among European larch (*Larix decidua*) and mountain pine (*Pinus mugo*) trees, which are widely distributed at high elevations. We investigated seasonal carbon isotope composition ($\delta^{13}C$) and concentration patterns of carbohydrates and organic acids in needles of these two different species from a case study in the Swiss National Park (SNP). We found that average and minimum air temperatures were the main climatic drivers of seasonal variation of $\delta^{13}C$ in sucrose and glucose as well as in concentrations of carbohydrates and citric acid/citrate in needles of both tree species. The impact of seasonal climatic drivers on larch and mountain pine trees at the needle level is in line with our earlier study in this region for long-term changes at the tree-ring level. We conclude that the species-specific changes in $\delta^{13}C$ and concentrations of carbohydrates and organic acids are sensitive indicators of changes in the metabolic pathways occurring as a result of climatic changes.

1. Introduction

Increasing temperature can cause a reduction of stomatal conductance because of increasing air to leaf vapor pressure deficit, while also influencing carbon dynamics via increased assimilation rates (Farquhar et al., 1989; Hu et al., 2010; Cernusak et al., 2013). Numerous studies demonstrated that limited water availability could lead to increasing isotope values ($\delta^{13}C$) in carbohydrates in needles of trees and reduce tree growth (Streit et al., 2013; Hartmann et al., 2013; Rinne et al., 2015). In contrast, low carbohydrate concentrations may sometimes occur as a result of stomatal conductance, photosynthesis and thus carbon limitation under drought (Wiley et al., 2012; Churakova (Sidorova) et al., 2018). However, an understanding of how carbon dynamics in needles of different tree species vary during the season and what kind of climatic information is recorded in these proxies at the compound-specific level under recent climate change is still scarce. In particular, sucrose is one of the dominating non-structural carbohydrates in tree leaves for carbon storage and as a form of transport metabolite in the phloem (Magel et al., 2000; Moing, 2000). Low amounts of sucrose could be caused by insufficient carbon supply due to a reduced transport function (Moing, 2000). Glucose and fructose are used for carbon storage, cell elongation and cryoprotection (Popp et al., 1997; Magel et al., 2000; Moing, 2000; Simard et al., 2013). Numerous studies demonstrated that sucrose and glucose concentrations in needles are increasing under low temperatures or limited water availability, i.e. under conditions of low carbon demand when growth is low (Streit et al., 2013; Lehmann et al., 2015; Rinne et al., 2015; Körner, 2015). Moreover, high carbohydrate concentrations may also protect against frost and water-loss under drought stress (Magel et al., 2000; Monig, 2000). Several studies have suggested that pinitol is essential for frost resistance (Sakai and Larcher, 1987) and may also be important for plants to endure drought stress (Monig, 2000; Rinne et al., 2015; Hoch et al., 2015).

Organic acids in plants, on the other hand, are involved in various

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anabolic and catabolic processes of metabolic pathways, which are essential for the primary metabolism, stomatal regulation, and the stored pools of fixed carbon compounds (Hettmann et al., 2005; Lehmann et al., 2016; Churakova (Sidorova) et al., 2018). One of the important physiological functions of organic acids is related to providing redox equilibrium, supporting ionic gradients on membranes, and acidification of the extracellular medium (Igamberdiev and Eprintsev, 2016). The major excreted organic acids are malate and citrate. Malate is often the most accumulated organic acid in plants and can contribute to the transfer of redox equivalents between cell compartments, playing a role as an osmolyte and anion for compensating the positive charge of potassium, which is particularly important in stomatal responses (Meyer et al., 2010; Geigenberger and Fernie, 2011; Maurino and Engqvist, 2015). The most abundant stored form of carbon can be citrate, depending on the intensity of reactions linked to conversion of this compound (Igamberdiev and Eprintsev, 2016), which is essential to iron acquisition and transport within the plant (Meyer et al., 2010). Hettmann et al. (2005) have shown that the biochemical production of malic and citric acids depends on species, environmental conditions (e.g., drought), and plant growth.

Climatic parameters like temperature, water availability, air humidity and ambient CO_2 influence photosynthetic CO_2 assimilation and water balance, which is reflected in the isotopic carbon composition ($\delta^{13}C$) of the plant organic matter, yielding a specific isotope pattern in the tree tissues. Under warm and dry conditions trees respond to limited water resources by reducing stomatal conductance, resulting in a diminished intercellular CO_2 concentration, and leading to a reduced ^{13}C discrimination (Farquhar et al., 1989).

Traditional use of bulk $\delta^{13}C$ isotope analysis of soluble carbohydrates has limitations in determining the responses of plants to environmental variations because compounds with slow turnover rates like pinitol can strongly dampen the signal (Rinne et al., 2015). More diverse information can be derived from individual compounds like pinitol, sucrose, glucose and fructose. So far, there are only a few studies on $\delta^{13}C$ for individual carbohydrates (Streit et al., 2013; Simard et al., 2013; Rinne et al., 2015; Smith et al., 2016; Churakova (Sidorova) et al., 2018) and organic acids (Hettmann et al., 2005; Lehmann et al., 2015, 2016; Churakova (Sidorova) et al., 2018) applying Compound-Specific Isotope Analysis (CSIA) with high-performance liquid chromatography (HPLC). Further studies are needed at the tree level to understand changes of metabolic pathways and to reveal specific impacts of climatic and environmental factors on plant metabolism.

In this study we aim to (i) reveal climatic factors driving seasonal $\delta^{13}C$ and concentration dynamics of individual carbohydrate and organic acid compounds; (ii) distinguish differences and identify similarities between larch and mountain pine trees in the variability of $\delta^{13}C$ and concentration of seasonal patterns for the various compounds; and (iii) reveal if the impact of climatic factors remain the same in seasonal and long-term changes at this study site.

2. Material and methods

2.1. Study area

The study was carried out at the site Champlönch, which is located in the Swiss National Park (SNP), 46° 40′ 48" N, 10° 10′ 20" E, at 1959–1964 m a.s.l. The SNP has an area of 174.2 km^2 and is the largest UNESCO protected area of Switzerland. Mountain pine trees (*Pinus mugo*) cover ca. 73% of the forests in the SNP, while European larch (*Larix decidua* Mill.) and Swiss stone pine (*Pinus cembra*) cover ca. 11%. *Pinus mugo* subsp. *uncinata* and *P. mugo* subsp. *mugo* are light-demanding species, which do not exhibit particular soil preferences but need sufficient water availability (Bendel, 2006). *Larix decidua* is better adapted to cold climate conditions than *Pinus mugo* and may reach life spans of more than 500 years (Churakova (Sidorova) et al., 2016).

2.2. Sampling design

To reveal the impact of climatic factors on seasonal changes in $\delta^{13}C$ variability of carbohydrate and organic acid compounds, we selected four dominant pine (Photo 1a) and larch (Photo 1b) trees from a south- (S) and north-(N) facing aspect resulting in a total of 16 trees. The trees were visually assessed to be healthy and did not show any scars, stem or crown damages. For each tree, needles were sampled during the growing period in 2013. The S-facing aspect located at 1964 m a.s.l., with 35° slope steepness is characterized as sunny and dry. In contrast, the N-facing aspect located at 1959 m a.s.l. with 20° slope steepness is characterized as shady with moister soil. It is covered by denser ground vegetation compared to the S-facing aspect. Needles from larch and pine (approx. 200 mg per sample) from both aspects were collected biweekly on sunny days. Because on the first sampling day on 24 May 2013 no new needles from mountain pine trees growing on either (S, N) aspects were visible, we collected pine needles from 2012. During this date, larch needles were very small and thus were not collected.

All sampled needles were immediately stored in cooling boxes with ice blocks and delivered to a mountain hut close to the study site, where they were heated in a microwave for 90 s at 250 W to stop enzymatic and metabolic activities (Wanek et al., 2001; Rinne et al., 2012, 2015). Subsequently, the samples were delivered to the laboratory at the Paul Scherrer Institute, where they were oven dried for 24 h at 60 °C and grounded with a steel ball mill to a fine powder, which was used for all further analyses.

2.3. Climatic data

Our study site is characterized by a continental climate with average winter (December–February) air temperatures of -9.2 °C and average summer (June–August) air temperatures of 9.5 °C, and 910 mm annual precipitation observed from 1917 to 2013 at the Buffalora weather station (46°39′ N, 10°16′ E, 1968 m a.s.l.), ca. 12 km away from the study site. The growing period is rather short and lasts from end of May to early June until end of September to early October. Average annual and spring temperatures have increased by 0.5 °C and 1.0 °C respectively, while annual precipitation has decreased by 88.9 mm for the period 1990–2013 relative to 1917–1989.

Data for daily average (T_{av}), minimum (T_{min}), maximum (T_{max}), temperatures, precipitations, reference evaporation (RE), relative humidity (RH), vapor pressure deficit (VPD), and wind speed were used from the local Buffalora weather station (Fig. 1).

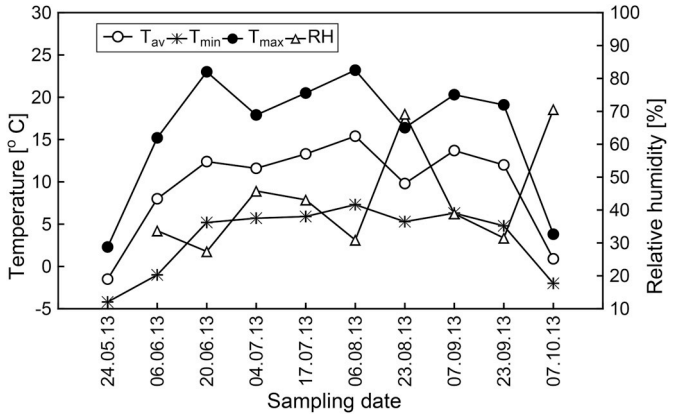

Fig. 1. Intra-seasonal dynamics of daily average (T_{av}), minimum (T_{min}) and maximum (T_{max}) air temperatures and relative humidity (RH) during the sampling in the growing period 2013.

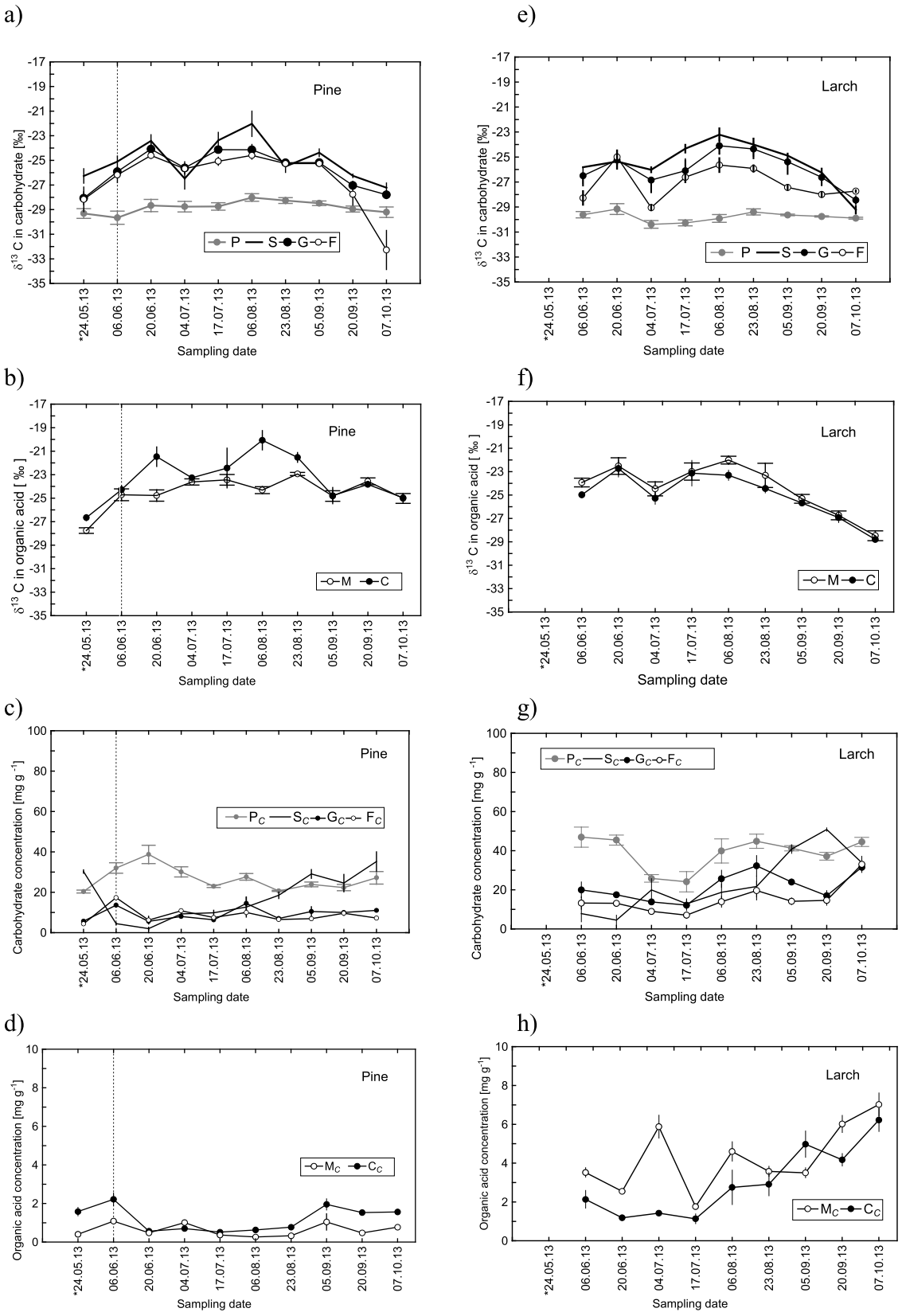

Fig. 2. Intra-seasonal dynamics of $\delta^{13}C$ of carbohydrates (P - pinitol, S - sucrose, G - glucose, F - fructose) (a, e) and organic acids (M - malate, C - citrate) (b, f); concentrations of carbohydrates (P_C - pinitol, S_C - sucrose, G_C - glucose, F_C - fructose) (c, g), and concentrations of organic acids (M_C - malate, C_C - citrate) (d, h) over the growing period 2013 for pine (a-d) and for larch (e-h) are presented. Error bars are based on ± 1 SE (standard error). *24.05.13 – mountain pine needles from the previous growth season of 2012 (vertical dotted line).

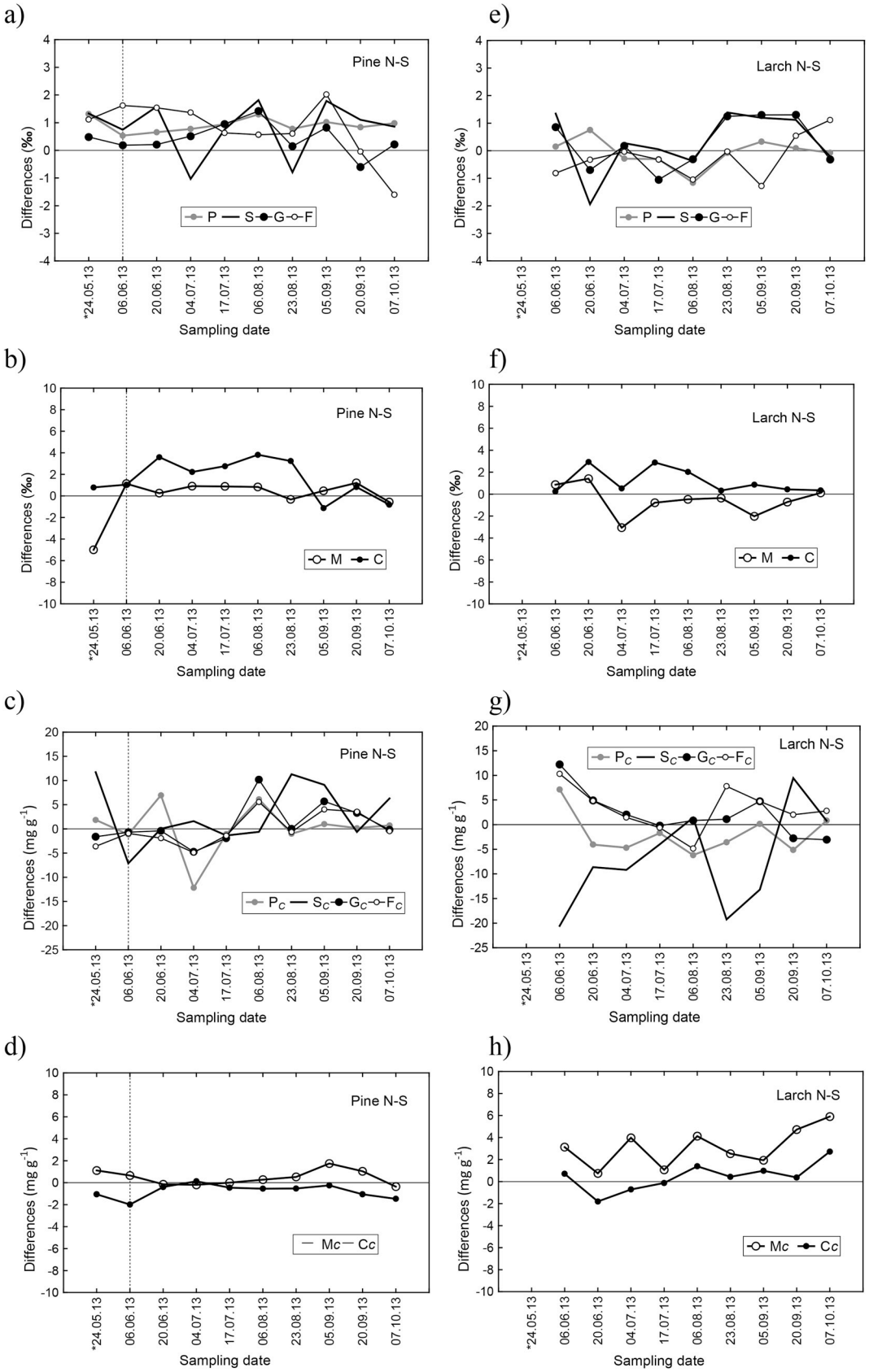

Fig. 3. Differences between north- (N) and south (S)- facing aspects calculated for each individual compound in needles of mountain pine (left panel) and larch (right panel) trees for: $\delta^{13}C$ in carbohydrates (P - pinitol, S - sucrose, G - glucose, F - fructose) (a, e); carbohydrate concentrations (P_C - pinitol, S_C - sucrose, G_C - glucose, F_C - fructose) (c, g); $\delta^{13}C$ in malate (M) and citrate (C) (b, f); and concentrations of malate (M_C) and citrate (C_C) (d, h). *24.05.13 – mountain pine needles from the previous growth season of 2012 (vertical dotted line).

2.4. Extraction and purification of sugars and organic acids

From the milled sample, 60 mg of powder was placed into Eppendorf tubes, 1.5 mL of Milli-Q water was added and then vortexed until the powder was fully suspended. The tubes were placed in the water bath at 85 °C for 30 min according to Wanek et al. (2001). The samples were cooled down for 30 min followed by centrifugation at 10 000 g for 2 min (Rinne et al., 2012, 2015; Churakova (Sidorova) et al., 2018).

For sugar purification, we used Thermo Scientific™ Dionex™ OnGuard II CRTG 1CC cartridges (USA): cation exchange cartridge "H" was used to remove amino acids, anion exchange cartridge "A" (HCO_3^-) to remove organic acids, and cartridge "P" to remove phenolic compounds present in the plant water extracts (Rinne et al., 2012). After sugar purification, we eluted organic acids from the cartridge "A" with 30 ml 1 M HCl solution, modified after Lehmann et al. (2015). Purified samples were stored at -20 °C until use.

2.5. Compound-specific $\delta^{13}C$ analysis

Compound-specific $\delta^{13}C$ analysis was performed on-line using a High-Performance Liquid Chromatography (HPLC) coupled via LC Isolink interface to a Delta V Advantage IRMS (both Thermo Fisher Scientific, Bremen, Germany). Soluble carbohydrates were separated on a Dionex CarboPac™ PA 20 3 × 150 mm column (Dionex, Olten, Switzerland) at 22 °C, using a 2 mM NaOH solution as the mobile phase with a flow speed of 250 µl min^{-1} (Rinne et al., 2012). The $\delta^{13}C$ CSIA analysis was performed for sucrose, glucose, fructose and pinitol. Fructose $\delta^{13}C$ and concentration can be affected by partial co-elution with other compounds. This is seen as a bigger deviation of fructose $\delta^{13}C$ and concentration values from those of glucose, and it prevents the use of fructose results in defining accurate compound-specific differences (Rinne et al., 2012).

Organic (malic and citric) acids were separated on an Allure Organic Acids 4.6 × 300 mm column (Restek, Bellefonte, USA) at 8 °C, using a 100 mM KH_2PO_4 (pH 3) solution as the mobile phase with a flow rate of 500 µl min^{-1} (Lehmann et al., 2015). Measurements of samples were interspersed by standards of different concentrations (20–180 ng C μl^{-1}), allowing for determination of concentration (via peak area) and for offset-correction of $\delta^{13}C$ values (Rinne et al., 2012). The overall measurement precision of δ^{13} C analysis of all standards was SD < 0.5‰.

2.6. Averaged and weighted $\delta^{13}C$ values of individual carbohydrate compounds

To make use of the advantage of compound-specific isotope analysis (CSIA) over bulk-analysis, we calculated weighted $\delta^{13}C$ averages on all the metabolites ($PSGF_W$), which represents bulk soluble carbohydrates based on our measurements from the $\delta^{13}C$ data of pinitol (P), sucrose (S), glucose (G) and fructose (F) [‰], and from the concentrations of pinitol (P_C), sucrose (S_C), glucose (G_C) and fructose (F_C) [mg g^{-1}] as:

$$PSGF_W = [P_C \cdot P + S_C \cdot S + G_C \cdot G + F_C \cdot F] / [P_C + S_C + G_C + F_C]$$

2.7. Statistical analysis

We applied Pearson correlation and ANOVA using the STATISTICA 12.0 software between climatic parameters, which were available from the Buffalora weather station and $\delta^{13}C$ values, and concentrations of individual carbohydrates and organic acids derived from larch and pine needles. Differences between north- (N) and south- (S) facing aspects were calculated for each individual compound in needles of mountain pine and larch trees based on subtraction of N- from S-aspect (Fig. 3).

3. Results

3.1. $\delta^{13}C$ values and concentrations of carbohydrates and organic acids in needles of larch and pine trees

We found a clear seasonal course of $\delta^{13}C$ and concentrations of individual carbohydrates in pine (Fig. 2a, c) and larch needles (Fig. 2e, g) during the sampling season in 2013. The $\delta^{13}C$ values of sucrose were less negative compared to other sugars for both species, showing a strong Pearson correlation with $\delta^{13}C$ values of fructose (r = 0.88; $P < 0.001$) and glucose (r = 0.99; $P < 0.001$) for pine and larch, respectively. The $\delta^{13}C$ of pinitol patterns in both larch and pine were rather invariable within the studied growing season (Fig. 2a, e). A depletion of ^{13}C of sucrose, glucose and fructose in both species started on 4 July 2013. The most pronounced differences between S- and N-facing aspects for both species were revealed for $\delta^{13}C$ in sucrose (Fig. 3e).

Seasonal patterns of carbohydrate concentrations in pine (Fig. 2c) and larch needles (Fig. 2g) differed significantly. Moreover, concentrations of pinitol and sucrose significantly differed between pine needles from S- and N-facing aspects (Fig. 3c), while for larch needles the difference was higher in sucrose (Fig. 3g). Concentrations of sucrose, glucose and fructose in pine needles were rather low at the beginning of the growing season, when maximum temperature reached only +2.3 °C (Fig. 1). Sucrose concentrations in larch needles (Fig. 2g) decreased by the end of the season. Such a decrease was not observed for glucose and fructose concentrations, showing a lower seasonal variability compared to sucrose concentrations (Fig. 2g). Pinitol concentrations in both pine and larch were generally higher compared to other carbohydrates during the growing season from early June until end of September to early October (Fig. 2c, g). Sucrose and pinitol concentrations in pine needles were significantly correlated (r = −0.60; $P < 0.001$), which was not the case for $\delta^{13}C$ values.

Generally, the seasonal pattern of organic acids was similar to the pattern of carbohydrates in both species, with ^{13}C enriched malic and citric acids at the beginning (early June) and depleted at the end of the growing season (early October) with clear differences between pine (Fig. 2b) and larch (Fig. 2f). The $\delta^{13}C$ values of malate and citrate in larch needles significantly correlated between each other during the growing season (r = 0.98; $P < 0.001$), which was not the case for pine needles. The differences between S- and N-facing aspects in $\delta^{13}C$ values of malate and citrate were more pronounced for pine needles (Fig. 3b) than for larch (Fig. 3f), with clear divergences in pine needles for the previous season 2012 (Fig. 3b).

Malate and citrate concentrations in pine needles (Fig. 2d) and their seasonal variability were lower compared to larch needles (Fig. 2h). A progressive increase of malate and citrate concentrations in larch needles (Fig. 2h) with the highest values at the end of the growing season was observed. Differences in seasonal variation between S- and N-facing aspects were more pronounced for malate concentration in larch needles (Fig. 3h).

3.2. Climate impact

Temperatures of daily average (T_{av}), minimum (T_{min}) and maximum (T_{max}) (Fig. 1) were significantly correlated with sucrose (S), glucose (G) and total amount of carbohydrates (PSGF) in needles of pine (Fig. 4a, b, c, left panel) and larch (Fig. 4d, e, f, right panel) trees. Seasonal changes of T_{min} correlated significantly with $\delta^{13}C$ of pinitol in pine needles only (Fig. 4b). Moreover, T_{min} significantly correlated with malate and citrate in needles of pine trees (Fig. 4b). T_{av} and T_{max} significantly correlated with malate and citrate compounds in needles of larch trees (Fig. 4d, f). Reference evaporation (r = −0.72; $P < 0.001$) was significantly correlated with sucrose concentration of pine needles.

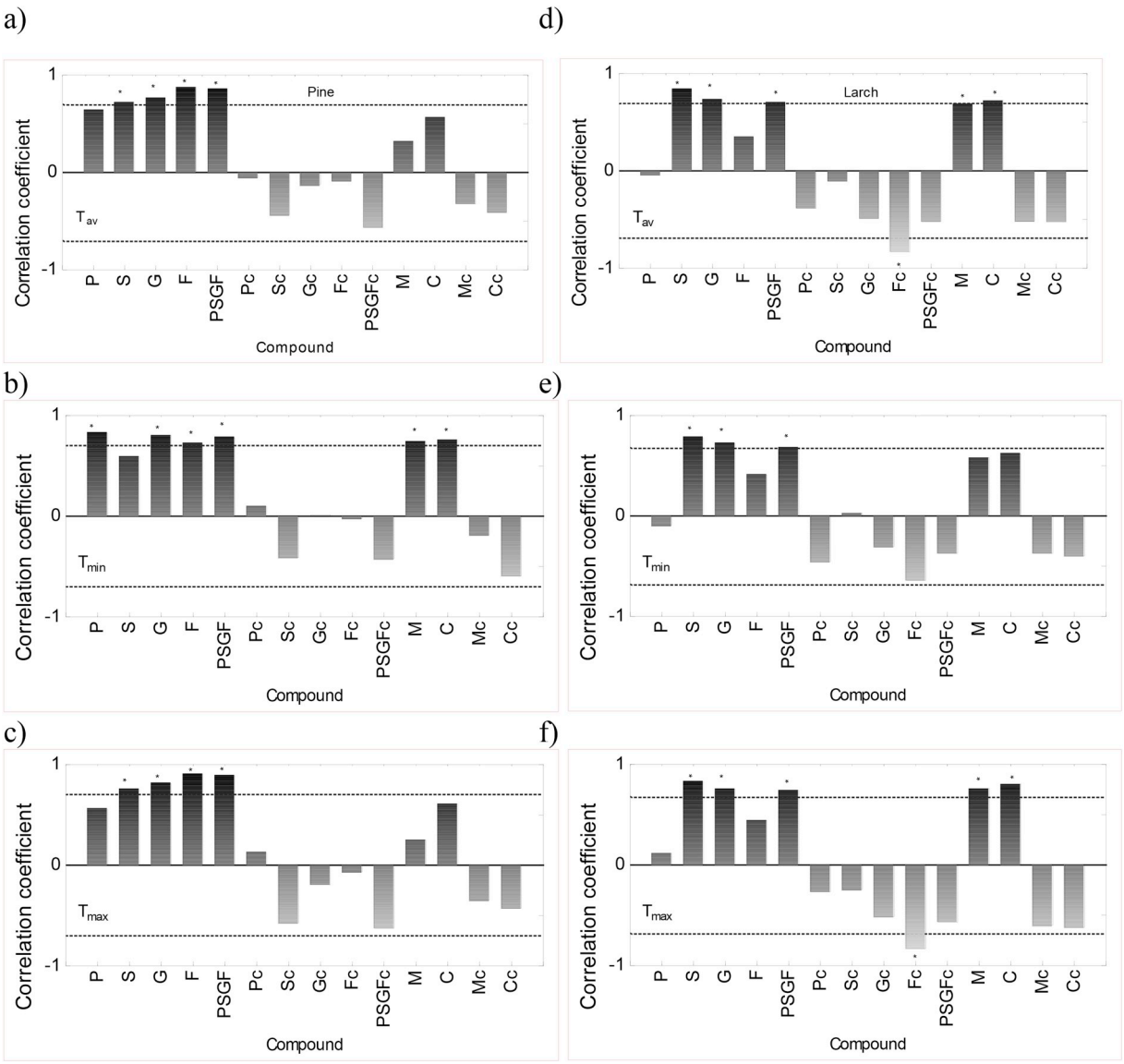

Fig. 4. Pearson's correlation coefficients calculated between average (T_{av}), minimum (T_{min}), maximum (T_{max}) air temperatures and $\delta^{13}C$ of carbohydrates (P - pinitol, S - sucrose, G - glucose, F - fructose, PSGF - averaged carbohydrates) and organic acids (M - malate, C - citrate); concentrations of carbohydrates (P_C - pinitol, S_C - sucrose, G_C - glucose, F_C - fructose, $PSGF_C$ - averaged carbohydrates), and concentrations of organic acids (M_C - malate, C_C - citrate) in needles of pine (a-c) and larch (d-f) trees. Asterisks present significant values $P < 0.01$.

3.3. Averaged seasonal patterns

The difference between average seasonal $\delta^{13}C$ values for pinitol between pine and larch (Fig. 5a) was almost 1‰, in contrast to the other compounds, where hardly any significant differences were found between the two species. The average pinitol concentrations showed clear differences between pine (26.5 ± 1.8 mg g^{-1}) and larch (38.9 ± 1.1 mg g^{-1}) needles (Fig. 5b), which were also higher than for sucrose in both species. $\delta^{13}C$ in malate did not vary significantly compared to citrate (Fig. 5c). Both malate and citrate concentrations showed significant differences (Fig. 5d) between the two species.

4. Discussion

4.1. Differences in seasonal $\delta^{13}C$ and concentrations in needles of larch and pine trees

We found that the seasonal variability of carbohydrates and organic acid concentrations to be clearly lower in pine needles compared to larch, which indicates lower carbon assimilation rates. This pattern can also be partly explained by species-specific distribution of leaf assimilates, which corresponds to a lower photosynthetic capacity in pine needles compared to larch. This is in line with the measurements in mountain pine and European larch (Streit et al., 2013) at a cold Davos site above timberline in Switzerland. Rinne et al. (2015) also showed a high seasonal variability in $\delta^{13}C$ of carbohydrates in needles of larch trees growing under harsh climatic conditions in Siberia and rather constant values of $\delta^{13}C$ in pinitol and insignificant correlations with other carbohydrates. Our study showed a similarly reduced seasonal variability of $\delta^{13}C$ in pinitol for both larch and pine needles. Differences in sugar concentrations of pine needles from the current and previous year in our study are in line with the discrepancies in cold hardiness between needles of different age classes (Schaberg and DeHayes, 2002). The dynamics between the “old” carbon pool and “new” freshly produced assimilates has the potential to influence $\delta^{13}C$ in bulk needles, which needs to be considered in evaluating the response of trees to

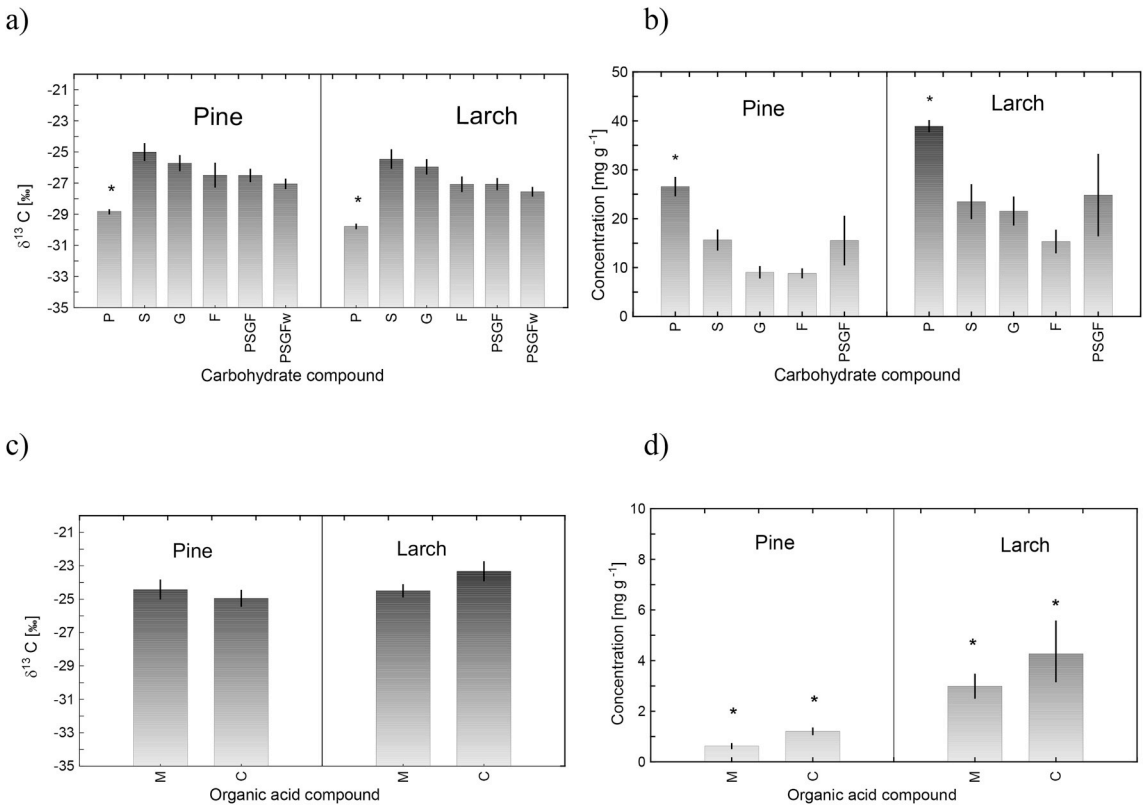

Fig. 5. Averaged seasonal $\delta^{13}C$ of carbohydrates (P - pinitol, S - sucrose, G - glucose, F - fructose, PSGF - averaged and weighted carbohydrates - $PSGF_W$) (a); Concentration of carbohydrates (P - pinitol, S - sucrose, G - glucose, F - fructose, PSGF - averaged carbohydrates) (b); $\delta^{13}C$ in organic acids (M - malate, C - citrate) (c), and concentrations of organic acids (M- malate, C - citrate) (d) in needles of pine and larch trees over the growth season of 2013 in comparison. Error bars are based on ± 1 SE (standard error). Significant differences between pine vs. larch trees are marked by asterisks *($P < 0.01$)*.

environmental changes. The stored forms of fixed carbon, malic and citric acids prevail in needles of larch trees. Concentrations of malate and citrate in needles of pine trees are rather low compared to larch trees and could be explained by reduced stomatal regulation and lower respiratory activity in pine.

4.2. Impact of climate drivers to seasonal variability of $\delta^{13}C$ and concentrations

In our study we found that mainly temperature plays a significant role for the seasonal variability of $\delta^{13}C$ in sucrose and hexoses, and concentrations in needles of both larch and pine trees. The main difference between the response of individual needle compounds to

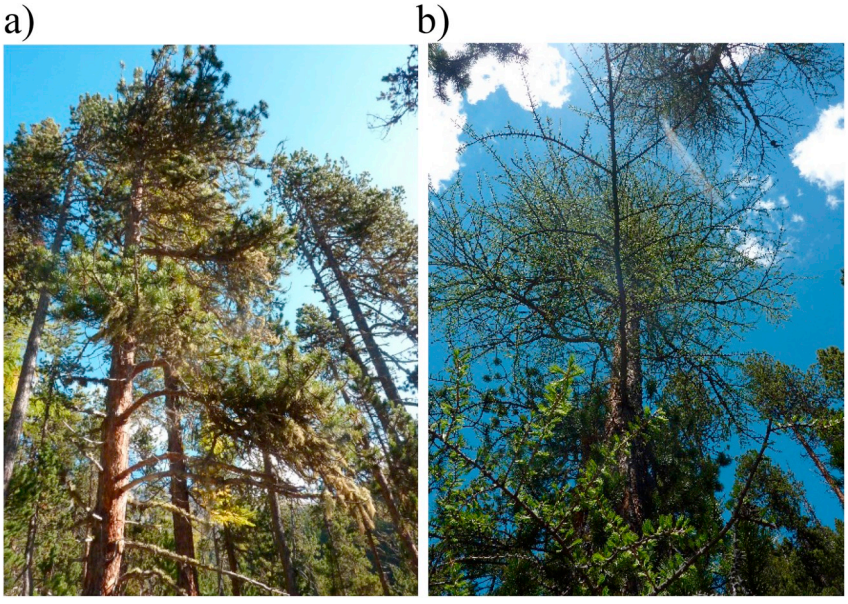

Photo 1. Dominant mountain pine (*Pinus mugo*) (a) and European larch (*Larix decidua*) (b) trees from the study site in the Swiss National Park.

climate parameters is, however, in the relationships between minimum temperatures and $\delta^{13}C$ of pinitol, and between sucrose concentration and reference evaporation, which is captured in pine needles only.

We found increasing $\delta^{13}C$ values for sucrose, glucose and fructose which was maintained until the end of July in needles of larch, while a longer increasing trend was found for mountain pine (Fig. 2). On the one hand this can be related to higher summer temperatures and vapor pressure deficit influencing the isotope fractionation during photosynthesis, but on the other hand also to post-photosynthetic processes. Sudachkova et al. (2004) showed that the need for reserve compounds in conifers was stronger at the beginning and at the end of the growing period, when the processes of xylogenesis had ended and when the carbon reserve pools are replenished, while glucose is converted to starch compound. Moreover, larch trees needed more reserve compounds for brachyblast needle formation in spring (Sudachkova et al., 2004). Differences in carbon reserves and carbon use therefore likely define the differences between deciduous larch and evergreen mountain pine under recent climate change.

4.3. Impact of climatic factors from leaf to tree-ring level

Our earlier study on $\delta^{13}C$ from wood of the same larch and pine trees over the period from 1900 to 2014 showed that both species were sensitive to temperature changes in June and July, while relative humidity mainly influenced pine trees (Churakova (Sidorova) et al., 2016). At the biogeochemical compound-specific leaf level we revealed that mainly the average and minimum temperatures were significantly associated with the $\delta^{13}C$ of sucrose, glucose, total amount of carbohydrates and both malic and citric acids in needles of both larch and mountain pine trees. Correlations between minimum temperatures and seasonal changes of $\delta^{13}C$ in pinitol and citrate in pine needles only suggest insufficient water availability for pine trees at this cold study site. Our earlier study (Churakova (Sidorova) et al., 2016) showed that intrinsic water use efficiency (iWUE) calculated for mountain pine trees continuously increased over the past decades, while iWUE calculated for larch reached saturation, possibly showing an adaptation to elevated CO_2. This could be explained by the plasticity of larch trees to improving growth conditions, and non-limiting water supply for larch (deep rooting tree) compared to mountain pine. Climate sensitivity from seasonal compound-specific leaf level is unchanged, reflecting similar strategies on different time-scales (Churakova (Sidorova) et al., 2016).

4.4. Averaged seasonal patterns

Our study is in agreement with the findings by Streit et al. (2013), who found a pinitol concentration of 39 ± 5 mg g^{-1} for larch (*Larix decidua*) and Scots pine (*Pinus sylvestris*) trees growing at a high-elevation research site in Stillberg near Davos (Switzerland). The authors also reported similar concentration values for sucrose. The differences between the trees of the two sites could be explained by the higher elevation of the study site at Stillberg (2180 m a.s.l.) and the colder conditions for the pine and larch trees investigated by Streit et al. (2013). In general, the carbohydrate concentration in pine needles was lower compared to larch needles over the whole season, which could be explained by different net photosynthetic rates. Streit et al. (2013) showed that mountain pine assimilated CO_2 at a significantly lower rate than larch growing at the same elevation and site.

5. Conclusion

(1) Seasonal variabilities of carbohydrate and organic acid concentrations were clearly lower in needles of evergreen mountain pine trees, which indicates reduced carbon assimilation rates compared to needles of deciduous European larch trees. This is plausible, as larch trees must produce new assimilation organs (needles) before it can gain new carbon, which is in contrast to pine trees.
(2) Mainly the average and minimum temperatures were significantly associated with the $\delta^{13}C$ of sucrose, glucose, total amount of carbohydrates and citric acids in needles of both larch and mountain pine trees. Correlations between minimum temperatures and seasonal changes of $\delta^{13}C$ pinitol and citrate in pine needles only suggest insufficient water availability for pine trees at this cold study site.
(3) Climate sensitivity from seasonal leaf level to long-term tree-ring level showed unchanged strategies. Long-term $\delta^{13}C$ variability in wood showed an impact of summer temperature for both species along with the impact of precipitation and VPD changes for pine trees.
(4) Our results showed that pine trees in the Swiss National Park are suffering from insufficient water availability, while larch trees showed the potential for adaptation and survival under recent climatic changes.

Declarations of interest

None.

Contribution

O.C(S)., C.B., M.S., R.T.W.S. planned and designed the research. O.C(S)., C.B., M.F., M.S., R.T.W.S. performed experiments, O.C(S)., C.B., M.F., G.T., conducted fieldwork, O.C(S)., C.B., L.S., M.M.L. analysed data and O.C(S)., M.S., M.M.L., R.T.W.S., C.B. wrote the manuscript.

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